

AFFINITY SEPARATION PROTOCOL

Construct affinity experiment - Talon-Co²⁺ matrix

SOLUTIONS

Lysis buffer

- 20 mM sodium phosphate
- 100 mM NaCl
- 20 mM imidazole

Binding buffer, pH 7.4

- 50 mM sodium phosphate, NaH₂PO₄
- 300 mM NaCl

Washing buffer, pH 7.4

- 50 mM sodium phosphate
- 300 mM NaCl
- 5 mM imidazole

Elution buffer, pH 7.4

- 50 mM sodium phosphate
- 300 mM NaCl
- 150 or 300 mM imidazole

8 M of Urea was added for denaturing conditions.

PROCEDURE

- 1) Remove the snap-off end at the Talon-Co²⁺ matrix column outlet and cap. Pour of the Ethanol solution.
- 2) Wash out the ethanol with 5 column volumes (CV) of distilled water.
- 3) Equilibrate the column with at least 5 CV of binding buffer.

Blank run: for first use of column

- a) Wash the column with 5 CV of distilled water.
- b) Wash with 5 CV of elution buffer.
- c) Equilibrate with 10 CV of binding buffer.

- 5) Apply rHER2-His6 suspended in washing buffer → Collect flow-through
- 6) Wash with 2 CV wash buffer → Collect sample
- 7) Apply lysate in washing buffer (Construct and HER2 amount should be equimolar) → Collect flow-through
- 8) Wash with 2 CV wash buffer → Collect sample
- 9) Elute with 2 CV elution buffer → Collect fractions